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THE ORNL SPACE BIOLOGY PROGRAM

ANNUAL REPORT

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BIOLOGY DIVISION

THE ORNL SPACE BIOLOGY PROGRAM

Annual Report,

Period Ending June 30, 1963

Prepared by

G. E. Stapleton and M. A Bender

The Basic Supporting Program, which involves estimation of the effectiveness of high-energy protons is one phase of research at ORNL, carried out under NASA Order Number R-77. The Biosatellite research is carried out at ORNL under NASA Order Number R-60.

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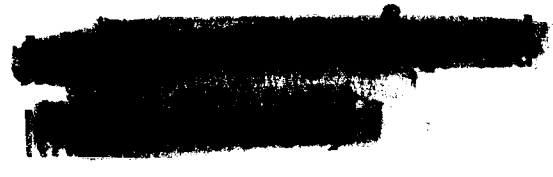

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SUMMARY

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This report describes work performed to July 1, 1963 in the ORNL Space Biology Program through an interagency agreement between the U.S. Atomic Energy Commission and the National Aeronautics and Space Administration. The investigations are divided into two chief categories: (1) a Basic Supporting Program, which includes the ground-based laboratory research chiefly concerned with the relative biological effectiveness (RBE) of high-energy protons on a variety of biological materials ranging from bacteria to human blood cells, and (2) a Biosatellite Program, which involves the development of testing methods and base-line radiation effects data on a number of biological materials for experiments in space vehicles in flight.

To date, the necessary equipment and instrumentation has been developed for proton irradiations, and experiments have been done with 22-Mev and 130-Mev protons. The results of these experiments are reported here. Plans for the future work with ground-based accelerators are discussed.

We have participated in two of the NASA in-flight programs, the BIOS I space probe and the 1962 Goose Bay high-altitude balloon flights. The biological experiments flown, and the experience gained from participation in the flights, are discussed. A series of experiments have been designed for the NASA Biosatellite Program, and a limited number of feasibility tests have already been performed. These experiments are also discussed.

Author

I. INTRODUCTION

The Biology Division of the Oak Ridge National Laboratory has two chief roles in the U.S. Space Sciences Program. It was decided in 1961 that the most important contributions this Division could make to the Program would be to assess the importance of the radiobiological aspects of space flight as well as the possible additive or synergistic action of the attendant physical factors, such as weightlessness and vibration, with low doses of radiation.

The program in the Biology Division was divided into two main projects: (1) the assessment of the relative biological effectiveness (RBE) of protons of various energies in a systematic series of biological materials ranging from microorganisms to human cells, and (2) the development of in-flight experiments for satellites which could answer the question of whether there are, in fact, any synergistic effects of radiation and the other mechanical and physical parameters encountered in space flight.

A. PROTON

It was realized from the outset that a systematic investigation of proton damage to biological materials could not be carried out using only the facilities at ORNL. A number of accelerators throughout the nation would be required on a cooperative basis. The following facilities seemed most appropriate, both because they produce external beams of protons spanning a wide range of energies and because they already have been used at one time or another for biological investigations.

1. ORNL - 86-in. cyclotron - 22 Mev protons[1]
2. Harvard University - 160-in. cyclotron - 160 Mev protons[1]
3. University of Chicago - 170-in. cyclotron - 440 Mev protons[1]
4. University of California - 184-in. cyclotron - 730 Mev protons[1]

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[1] Full energy of the beam. The energy is somewhat degraded at the site of biological exposures.

In addition, the Oak Ridge Isochronous Cyclotron (ORIC) will soon supply 40-80 Mev protons, and the Heavy Ion Linear Accelerator (HILAC) at the University of California will be available for possible investigation of high-energy heavy ions. It is anticipated that ORIC will also be able to supply these particles in the future.

To date, only the first two listed facilities have been used. This report summarizes the investigations carried out at those locations.

B. BIOSATELLITE

Biology Division in-flight experiments are, naturally, dependent on the availability of suitable NASA flight vehicles. Two series of flights have so far been made available to the Biology Division, and experiments have been flown in both series. The first flights were the BIOS I series of two ballistic space probes fired from Point Arguello, Pacific Missile Range, in November, 1961. The second was the series of five high-altitude balloons flown from Goose Bay, Labrador, in July and August, 1962. The BIOS I flights were designed to penetrate the inner Van Allen radiation belts and to provide 28 minutes of weightlessness. The balloon flights were designed to provide roughly 2-1/2 days of exposure to cosmic-ray fluxes at an altitude of approximately 130,000 feet and at a latitude of about 53°N. Unfortunately, various launch, tracking, recovery, or life-support difficulties during both series prevented completion of the Biology Division experiments. Nevertheless, the experience in the problems of in-flight experimentation gained from participation in these series of flights was extremely valuable and is being used in the planning of experiments for the scheduled Biosatellite flights.

The Biology Division has participated in the planning of the NASA Biosatellite Program since its inception in late 1962. Special attention has been given to the design of the "Radiation Biosatellite" portion of this program. Five ORNL experiments have been incorporated into the planned radiation satellites. The philosophy used in designing the experiments, and consequently the satellite itself, is that in order to test properly the possibility of an interaction between radiation and any of

the parameters of the space flight itself the biological materials must be exposed to known doses of a known quality of radiation in orbit. This requirement dictates that the satellite carry its own (probably isotopic) radiation source. In addition, the satellite will carry properly shielded specimens that will test whether or not the ambient radiations encountered during the flight produce any unpredictable effects.

II. PROTON RBE PROJECT

A. BIOLOGICAL MATERIALS AND METHODS

1. Bacterial Inactivation and Mutation

G. E. Stapleton

The bacterium, Escherichia coli, is one of the most frequently used radiobiological test objects. The cells of this species can be rapidly and easily cultured. The techniques for preparation of resting cell populations are relatively simple. A large number of important aspects of radiation damage are quantitatively measurable with this organism, such as (a) lethal effects, (b) mutagenic effects, (c) repair or recovery, and (d) protection by physical and chemical means.

Cell inactivation and mutation can be measured simultaneously with the same cell populations, and moreover, mutations can be measured simultaneously at several genetic loci in the same irradiated cell population.

The E. coli populations are cultured to maximum stationary phase (18 to 24 hours at 37°C), washed free of the growth medium by centrifugation, and resuspended in physiological saline or a phosphate buffer (usually 0.066 M) at pH 6.8. Cells from such suspensions can be irradiated in suspension, in a monolayer on a thin membrane filter, or in a thin layer on non-nutrient solid agar. Under all three conditions the organisms respond to radiation in a similar manner. In suspension as many as 10^{11} cells/ml can be irradiated. On an agar surface approximately 10^{11} cells can be irradiated in a thin layer <1 mm thick. On membrane filters no more than about 10^{8} cells can be irradiated in a monolayer. For inactivation studies any of the techniques are used. However, for mutation studies 10^{9}-10^{11} cells must be irradiated to obtain reliable statistical evaluation since the spontaneous mutation frequencies range from 10^{-10} to 10^{-8} for most loci in many species.

For microbiological studies with the 22-Mev protons (range about 3 mm in H_2O or in bacteria) it was necessary to use the monolayer-filter or agar-surface technique. For the higher energy protons thick suspensions could be used.

Suspensions that contain a large number of cells (10^{10}/ml or more) in a small closed tube will become anaerobic in 30 minutes at 30°C. In experiments in which the effect of oxygen was measured, suspensions containing 10^{6} cells/ml or less were used. These do not become anaerobic under these conditions. In addition to investigation of the influence of gaseous atmosphere, cells in suspension at lower concentrations were incubated for 15 minutes before irradiation with 0.12 M β-mercaptoethylamine and irradiated to test chemical protection against these radiations.

Cells in thin layers on membrane filters or on agar surfaces which are suitable for low-energy proton experiments do not lend themselves readily to such measurements. The gaseous atmospheres in the whole irradiation sample changer must be controlled; this is true also for experiments involving chemical protection, since the most effective chemical agents are auto-oxidizable.

For most of the inactivation studies to be reported, E. coli B/r (ORNL) was used. For the mutation studies a mutant strain of E. coli (K-12) was used. This mutant strain, AB-531, is auxotrophic for several amino acids and carries a number of markers for sugar fermentation. Spontaneous and induced mutations were measured as revertants to nonrequirement for the specific amino acids or to ability to utilize the specific sugars as sole carbon source. The number of revertant cells is measured by plating cell populations on media not containing the required factor. The number of revertants obtained from unirradiated cell populations yields the spontaneous mutation frequency. The number obtained in irradiated populations, corrected for the number obtained in unirradiated populations, gives the induced frequency. It is common to express the frequency in terms of number of revertants per surviving cell. In low-dose experiments where little killing is obtained, there is no objection to expressing frequencies in this manner.

In summary, the following measurements are routinely made.

1. Inactivation - fraction of surviving cells as a function of dose.
2. The role of the gaseous atmosphere.
3. Chemical protection against inactivation.
4. Induced mutation frequency.

2. Inactivation and Mutation at a Specific Locus in Neurospora

F. J. de Serres B. B. Webber

In contrast to the bacterial system, in which mutation frequencies are measured by reversions or back mutations, it is possible to measure directly forward mutations in the mold Neurospora crassa over the same range of mutational events that occur in diploid organisms by use of heterokaryotic conidia (asexual spores). The genetic markers in each component of this heterokaryon (actually a dikaryon in which the two nuclei are genetically different) permit detailed analysis of the mutation events occurring in a specific region of the genome, the adenine-3 region.

Mutations in the ad-3 region cause a requirement for adenine and accumulation of a reddish purple pigment in the vacuoles of the mycelium. Thus, ad-3 mutants are colored and wild-type colonies are colorless. When treated conidia are incubated under the appropriate conditions, each one develops into a spherical colony 2 mm in diameter. When known amounts of a conidial suspension are incubated either untreated or after treatment with various mutagens, counts of the total number of colonies/unit volume of medium can be used to estimate survival, and the ratio of the total purple colonies to the total survivors can be used to calculate forward mutation rates. Over a wide range of treatment times or radiation exposures, such counts permit analyses of the kinetics of both conidial inactivation and mutation induction.

Mutation in the ad-3 region can involve the ad-3A or the ad-3B locus or both, and be either reparable or irreparable on adenine-supplemented medium. Heterokaryon strains made up with appropriate tester strains carrying mutations at closely linked loci can be used to determine the extent of the genetic damage in the ad-3 region. Present testers permit detection of the complete range of genetic events that might give rise to a purple colony in forward mutation experiments, ranging from single nucleotide pair substitutions at either locus to loss of the whole chromosome.

Since the ad-3 mutations are obtained on the basis of pigment accumulation, there is marked variation in the requirement for adenine among ad-3A and ad-3B mutants. The spectrum of requirements for adenine among samples of ad-3 mutants varies according to the mutagenic origin.

More detailed analysis of induced genetic effects may be obtained by an analysis of the gene products specified by mutants at the ad-3B locus. Heterokaryon tests with the appropriate ad-3B tester strains can be used to determine (1) the spectrum of complementation patterns, and (2) the percentage of mutants showing positive tests with any tester, in samples of mutants of different mutagenic origin. Both the percentage of mutants showing allelic complementation (giving positive tests with the ad-3B tester strain) and types of complementation patterns vary markedly as a function of mutagenic origin.

Experimental Procedure.--Known aliquots of suspension of conidia of a balanced dikaryon are collected on "Millipore" filters, dried over silica gel, and then packed in air-tight nylon containers. After treatment conidia are humidified in moist air and then resuspended in water before being incubated to permit assay of changes in survival and for mutation induction. Conidia are incubated with 1 X 10^6 survivors/10 L medium to permit heterokaryotic conidia to form colonies as described previously. After 7 days of incubation, colonies are harvested and counted.

The experimental measurements made are as follows:

1. Inactivation - dose-survival curves
2. Mutation - dose-induced mutation frequency determinations
3. Percentage of ad-3A, ad-3B, ad-3A-3B mutants
4. Percentage of viable and recessive mutations
5. Percentage of allelic recombination of ad-3B.

3. Killing of Spermatogonia and Oocytes in Mice

E. F. Oakberg Evalyn Clark

Certain cells of the mammalian testis and ovary are extremely sensitive to ionizing radiations. Gametogenesis in the male and the female and its dynamics are now sufficiently well understood to make a study of the degeneration of certain cell types a sensitive and reliable measure of radiation damage in mammalian cells. For the experiments to be reported here, male and female hybrid mice from the cross between 101 females and C3H males were used at 3 to 4 months of age. The mice have been subjected only to the 130-Mev proton beam to date, but will be irradiated also with

higher energy protons as these become available. Investigations have already been carried out with 250-kvp X rays and γ rays at several intensities with Co^{60} and Ce^{137} sources and with 14 and 2.5-Mev fast neutrons. For comparative purposes the earlier data will be referred to in the section on Results.

The mice were irradiated at various proton doses from 5 to 200 rads. Three days after exposure the animals were killed by cervical dislocation. The excised testes or ovaries were fixed in Zenker-formol and serial sections made for counting normal and degenerate cells. The ratio of normal cells in irradiated and unirradiated testes or ovaries was used to estimate cell survival as a function of dose. A control series was subjected to X rays at the same time to compensate for any stress which may have been induced by transportation of animals to accelerator locations.

4. Chromosome Aberrations in Human White Blood Cells

M. A Bender P. Carolyn Gooch

In these investigations the number of chromosome aberrations produced in samples of human blood was scored. The investigators are the blood donors in all experiments. Five ml of peripheral blood is withdrawn and heparinized, placed in a thin nylon capsule, and irradiated. After irradiation the serum and leukocytes are separated from the blood by centrifugation and incubated in tissue culture medium containing the lectin Phytohemagglutinin for 3 days at 37°C. Before fixation the cultures are treated with colchicine and hypotonic saline. Previous experiments with H^3-labeled thymidine have shown that the cells scored are from the first in vitro mitosis. The cells are scored by standard cytological techniques. The types of aberrations seen are deletions and ring and dicentric chromosomes. For comparative purposes the most useful estimates are based on the "coefficients of aberration production."

B. PHYSICAL METHODS

The experimental setups at the various accelerators have much in common. These include sample holders or changers, a device for measuring total current or flux, such as a Faraday cup, and tissue-equivalent dosimeters such as LiF. Except for the 22-Mev proton beam, some sort of dosimeter is also incorporated as a necessary part of the accelerator setup, such as a current integrator with an accessory counter. After a series of calibration with dosimeters, the usual technique is to base the exposure time on the current integrator.

1. 22-Mev Protons

a. Exposure Systems.--Since protons of this energy have a range of only a few millimeters in biological material or water, it was not feasible to incorporate a tissue-equivalent ion chamber in the beam. It was possible, however, to test the homogeneity of the beam by darkening of thin glass plates with a microdensitometer. With a 2-mil aluminum scattering foil placed upstream from the exposure location it was possible to have a homogeneous beam about 1 in. in diameter at the sample location. This beam was of sufficient area to permit the bacterial and fungal experiments. However, additional scattering material was required to spread the homogeneous portion of the beam to a diameter of 5 cm for irradiation of a sufficiently large blood sample (about 5 ml) with a thickness of 3 mm or less. Such a beam is being produced now and its homogeneity is being tested with glass plates and also with copper foil and silver-metaphosphate glass rod dosimeters.

Figure 1 is a view of the bacteriological sample changer and accessory electronic gear as used on the ORNL 86-in. cyclotron. There are two 12-position wheels, remotely controlled and motor-driven. The sample changer can be charged with any pressure of any desired atmosphere during irradiations. Some details of the sample changer are shown in Fig. 2, including the Faraday cup and gas port. Figure 3 shows the human blood sample changer in place on the beam pipe. It will be noted that the cross-shaped portion is common to both sample changers and is provided

with a NaI crystal detector at lower left. Above center of the tube is a positioning knob for locating a 1-mil aluminum scattering foil in the beam to scatter a small fraction of the protons to the scintillation detector as a secondary means of measuring the beam current. Details of construction of the blood-sample changer are shown in Fig. 4. This figure also shows a sample holder and the nylon blood capsule with its 0.25-mil Mylar cover.

b. Dosimetry.--No ion chamber is provided in the physical setup for 22-Mev protons. However, experiments are in progress with silver metaphosphate glass-rod and LiF thermoluminescent dosimetry in the containers to be used for human blood irradiations.

With a knowledge of the beam current or flux traversing the biological sample and of the stopping power of the material, it is possible to estimate the dose in rads. In addition, LiF is virtually tissue equivalent, giving direct rad doses.

Using a collimated beam of known dimensions as we have in the physical setup at the ORNL 86-in. cyclotron, we need only a measurement of the current (Faraday cup) and a knowledge of the relative cross-sectional areas of the beam and the biological samples it traverses to calculate doses for thin specimens such as bacteria and Neurospora.

2. 130-Mev Protons

a. Exposure Systems.--The primary beam, well focused by the quadripole magnets, is scattered in Lucite, which reduces the energy to about 130 Mev and makes the beam homogeneous over an area 4 cm in diameter. The collimated beam passes through a nitrogen-filled or helium-filled ion chamber provided with thin, aluminized Mylar windows before reaching the biological samples.

The maximum range of these protons is 17 cm in tissue. In the experiments to be reported here the greatest sample thickness was 2 cm or so; therefore, the Bragg peak did not occur within any of the samples.

Since the physical irradiation setup is already so well developed, it was necessary only to provide the simplest of sample holders; in most

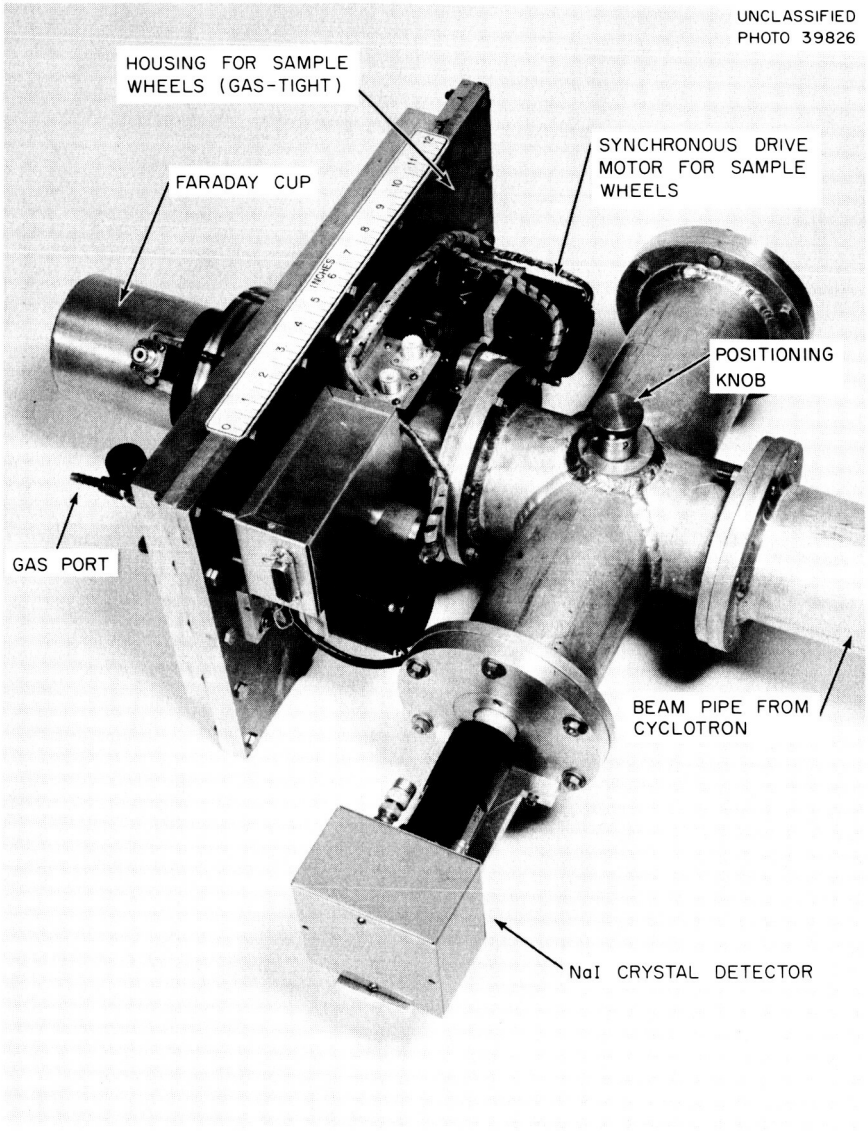

Fig. 1. Sample changer and equipment for microbiological investigations in the ORNL 86-in. cyclotron. The graphite shutter is not shown. The crystal detector is for scattered protons, the positioning knob for the 45° aluminum scattering foil, and the gas port is used for evacuation or changing atmosphere in wheel housing.

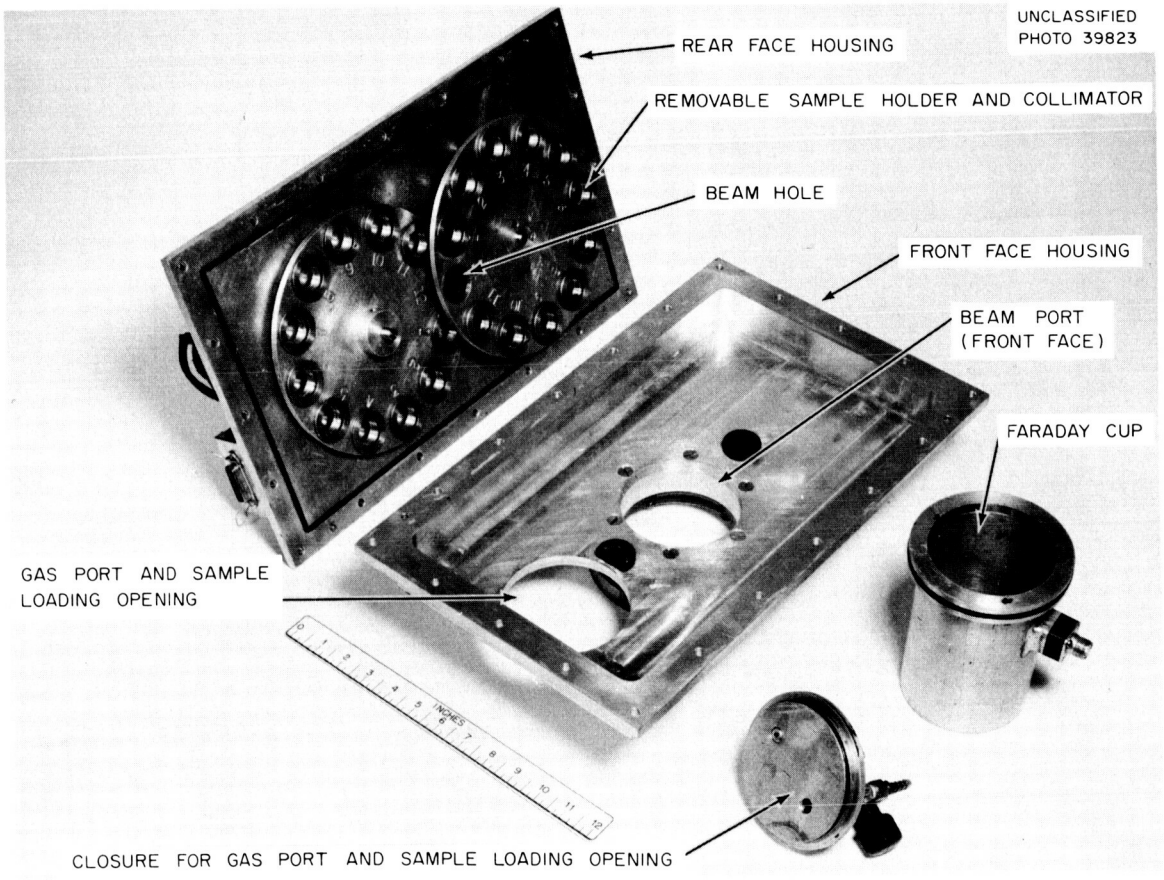

Fig. 2. View of disassembled wheel housing.

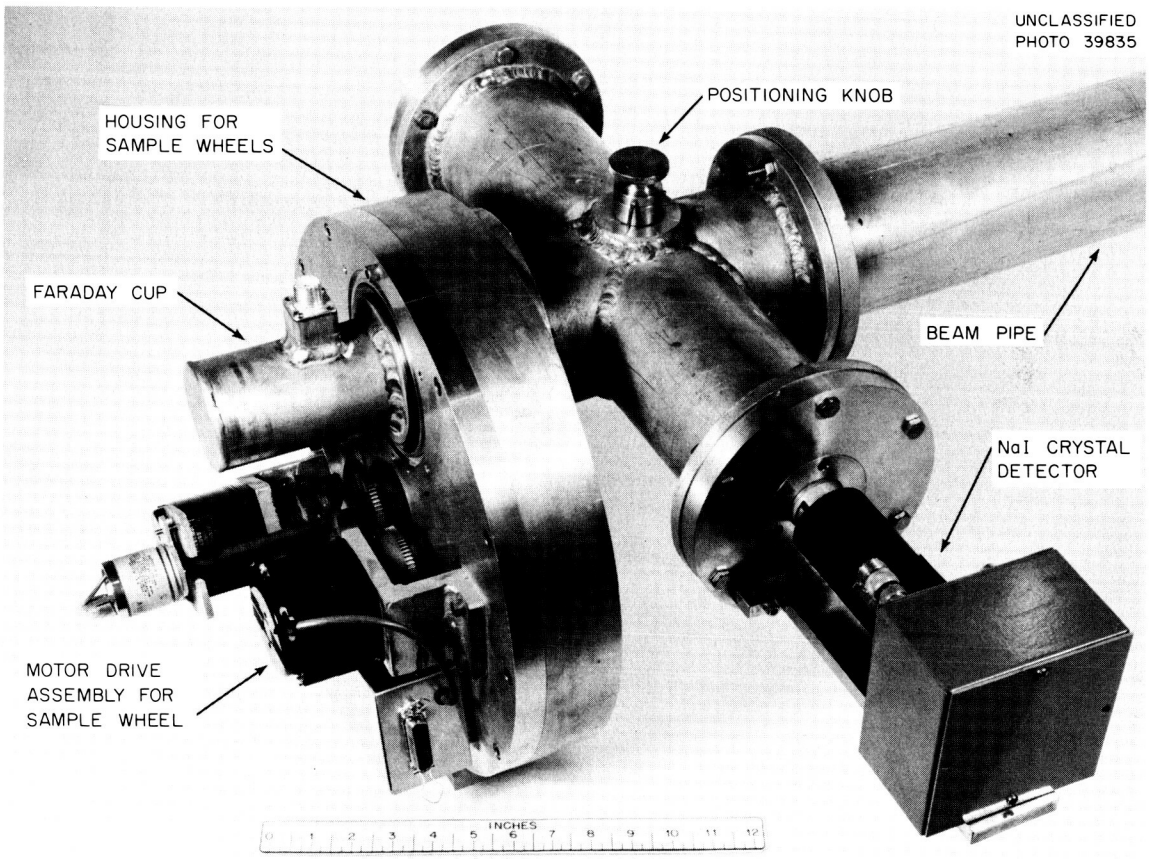

Fig. 3. Sample changer for human blood irradiation in the ORNL 86-in. cyclotron. See Fig. 1

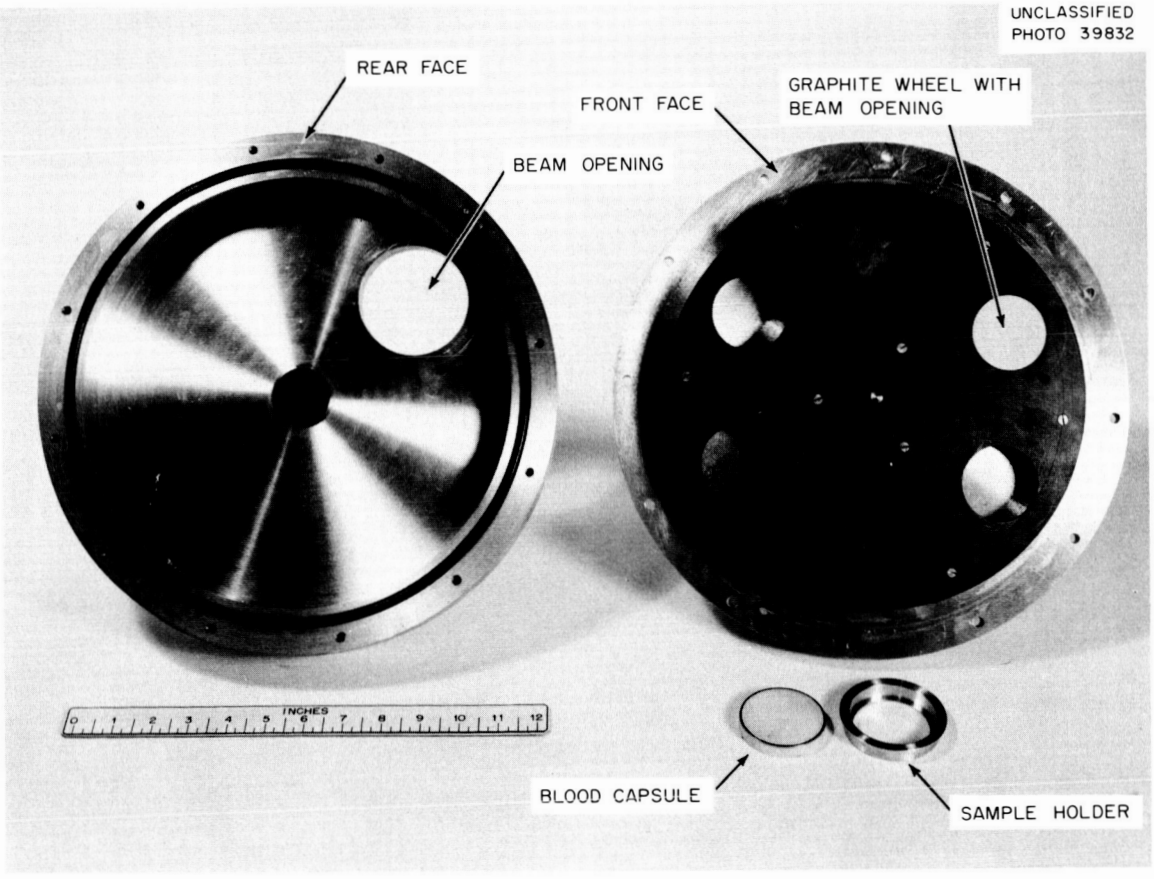

Fig. 4. View of disassembled wheel housing of blood sample changer.

cases this permitted the irradiation of one or two samples per irradiation. No elaborate sample changers were used. The type of sample holder used for microbiological samples is shown in Fig. 5. To minimize production of secondary radiation all sample holders were of plastic, either cellulose acetate or nylon (for containers that required sterilization).

The dose rates used in all experiments approximated those used in base-line X-ray exposures: 100 rad/min for the mammalian samples and 2,000 rad/min for the microbiological samples.

b. Dosimetry.--Since this facility is used routinely for irradiation of mammals as well as of human patients, the dosimetry is well developed.

3. Other Accelerators

The proposed experiments with higher energy protons have not as yet begun, but arrangements have been made to complete the series up through 730-Mev protons in FY-1964.

C. RESULTS FOR FY-1963

1. Experiments with 22-Mev Protons

The ORNL 86-in. cyclotron had to be adapted to use for biological exposures, having been built primarily for high-current internal beams. The beam currents required for biological experiments are 10^{-3} to 10^{-4} those used routinely for internal target irradiations. Moreover, the necessary beam cross-sectional areas for biological experiments are many times those usually desired in physical experiments. Means of scattering the beam or defocusing it were necessary, as were means of monitoring the unusually low intensity beam. When stable operation at low beam current is achieved it is also necessary to shutter the beam and to change biological samples by remote control. Facilities for doing this have been provided in the present biological facility.

a. Inactivation and Mutation in E. coli.--This facility has only

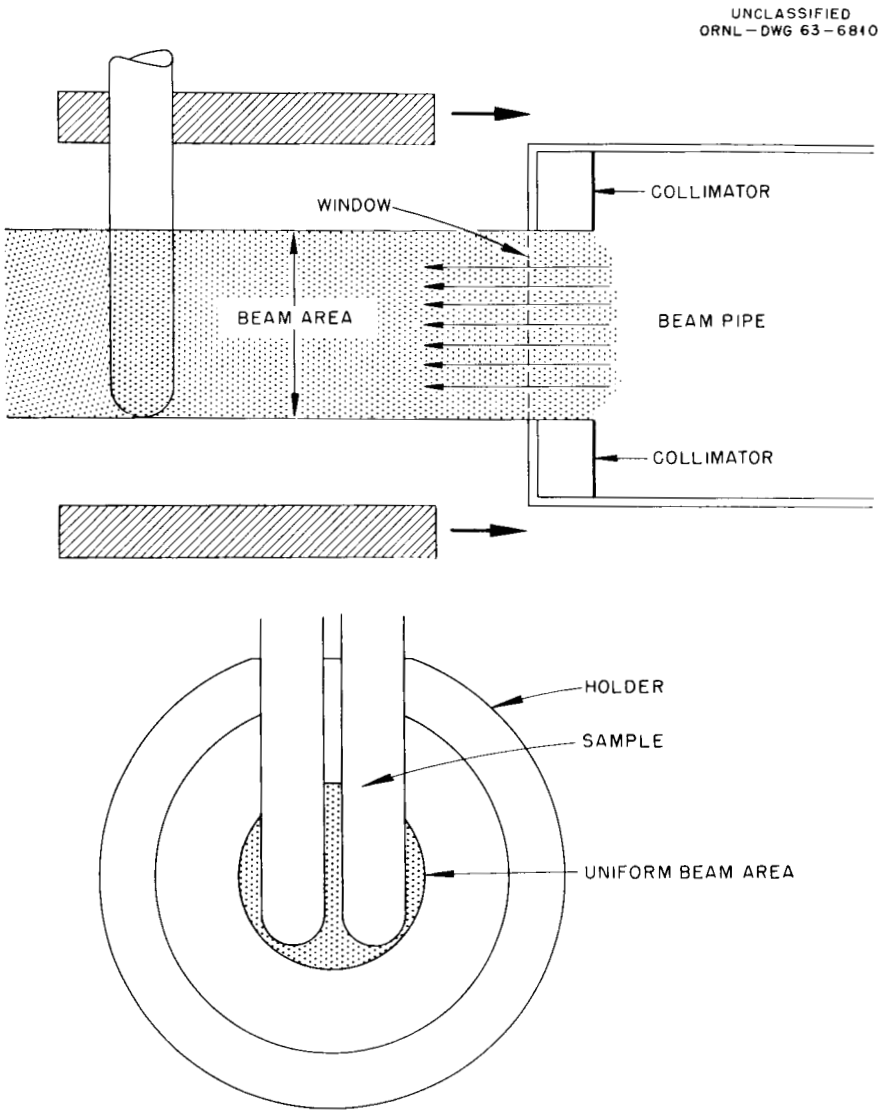

Fig. 5. Diagrammatic view of sample holder (microbiological) for 130-Mev protons.

just become available for routine biological irradiations. Nevertheless, a series of bacterial inactivation experiments have already been carried out. The results are shown in Fig. 6. The surviving fraction of cells is plotted as a function of proton and X-ray dose in kilorads. Each point is the average of six runs. Irradiations have been carried out so far with bacterial cells in a monolayer on membrane filters backed by soft nonnutrient agar. The comparison of sensitivity of these cells irradiated with 250-kvp X rays, either in suspension in equilibrium with air or on membrane filters in equilibrium with air, shows no significant difference. However, from the data described in Fig. 6 the protons seem to be somewhat less effective per unit dose. (RBE at LD-90 or LD-99 is about 0.8.)

As will be seen in other experiments with 130-Mev protons, the same sort of RBE was obtained for bacteria irradiated in equilibrium with air.

Experiments involving irradiations in air, in an oxygen-free atmosphere, or in the presence of the chemical protector, MEA, are under way.

b. Inactivation and Mutation in Neurospora.--Several preliminary inactivation curves have been made with heterokaryons of Neurospora crassa. Although the data are fewer than those for bacteria, the same general tendency for a lower RBE for 22-Mev protons than for 250-kvp X rays was found.

Further experiments are in progress and data on both inactivation and mutation induction will become available during early FY-1964.

c. Chromosome Aberrations in Human Blood Cells.--As mentioned previously the homogeneity of the scattered beam is now being measured by use of small copper foils, silver-metaphosphate glass rods and glass plates, placed over the biological sample holders. Also, dosimetry within the sample holder is being done by use of both silver-metaphosphate glass rods and LiF thermoluminescent dosimeters. No actual exposures of human blood samples will be made until the physical measurements are completed.

The short range of the 22-Mev protons of course precludes total-body irradiation of whole mammals.

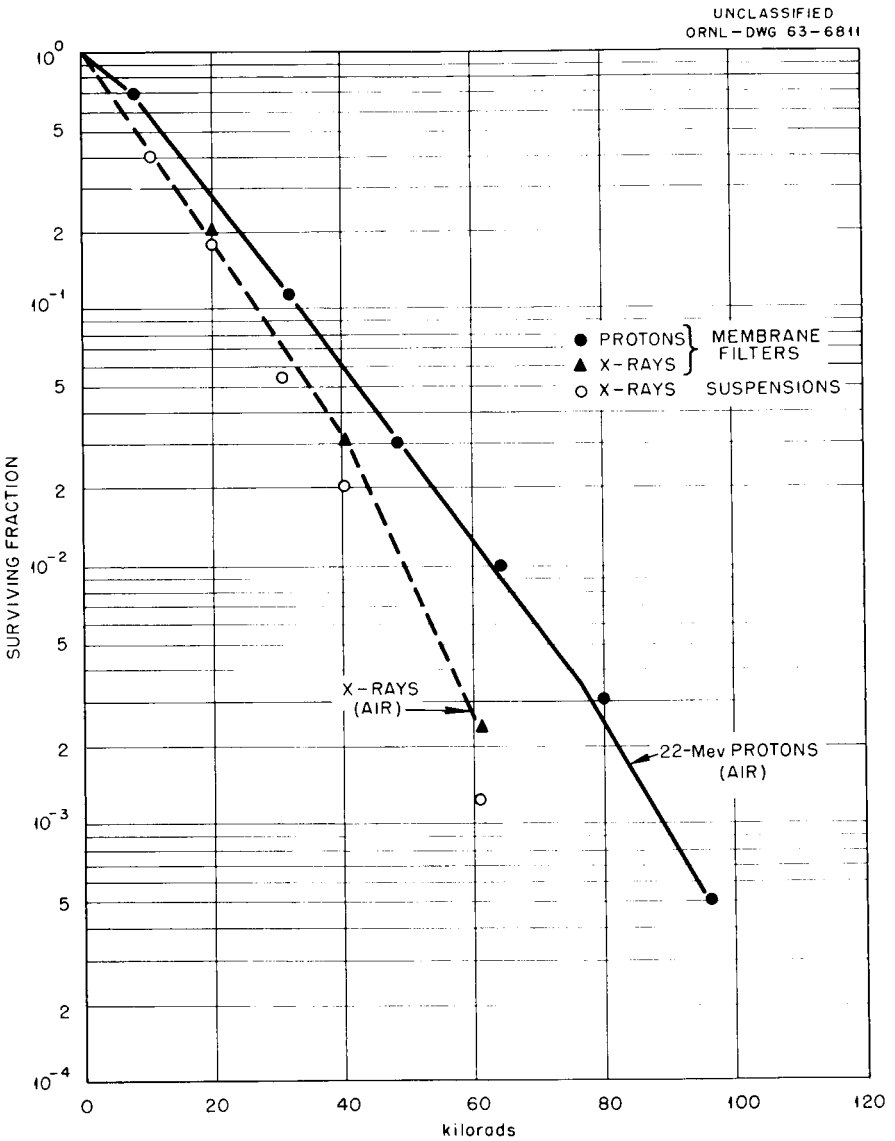

Fig. 6. Inactivation of E. coli B/r (ORNL) by irradiation with X rays and 22 Mev protons.

2. Experiments with 130-Mev Protons

Experiments in which 130-Mev protons were used are more complete than those just described for lower energy protons.

a. Inactivation and Mutation in E. coli.--Cells of E. coli B/r (ORNL) were irradiated in suspension in 0.066 M phosphate buffer at pH 6.8: (1) at concentrations of 2×10^6 cells/ml in equilibrium with air, (2) at the same cell concentration in buffer solutions of 0.12 M MEA, and (3) in tightly closed tubes at a cell concentration of 2×10^{10} cells/ml. The suspensions were irradiated with 250-kvp X rays and 130-Mev protons under the three conditions described, and the results are shown in Fig. 7. It is of interest that with suspensions in equilibrium with air an RBE (proton/X ray) of ~0.8 was obtained, similar to that found for 22-Mev protons. However, in the absence of air or oxygen and in the presence of MEA, an RBE of 1.0 was obtained with the 130-Mev protons.

Figure 8 shows data for inactivation and mutation induction for two loci in E. coli (AB-531). Since the suspensions irradiated with protons contained a high concentration of cells (10^{10} cells/ml), it is assumed that they are anaerobic. The X-ray controls, aerobic and anaerobic, are shown for comparison. The mutation frequencies for protons are from a single experiment and the data involve large errors. Nevertheless, the RBE for inactivation and for mutation approximate 1.0, as would have been predicted from experiments with B/r and also from other recently published data.

b. Killing of Mouse Spermatogonia and Oocytes.--A series of 33 mice, both male and female, was irradiated with 130-Mev protons at doses from 5 to 200 rads. A comparable series was subjected to 250 kvp X rays. The animals were killed 72 hours later and the testes and ovaries fixed, embedded, and sectioned. The number of spermatogonia and oocytes killed was determined by counts on stained sections. The data are presented in Tables 1 and 2.

As in the case of the previously reported data on bacterial inactivation, an RBE somewhat lower than 1.0 was observed for both oocytes and spermatogonia.

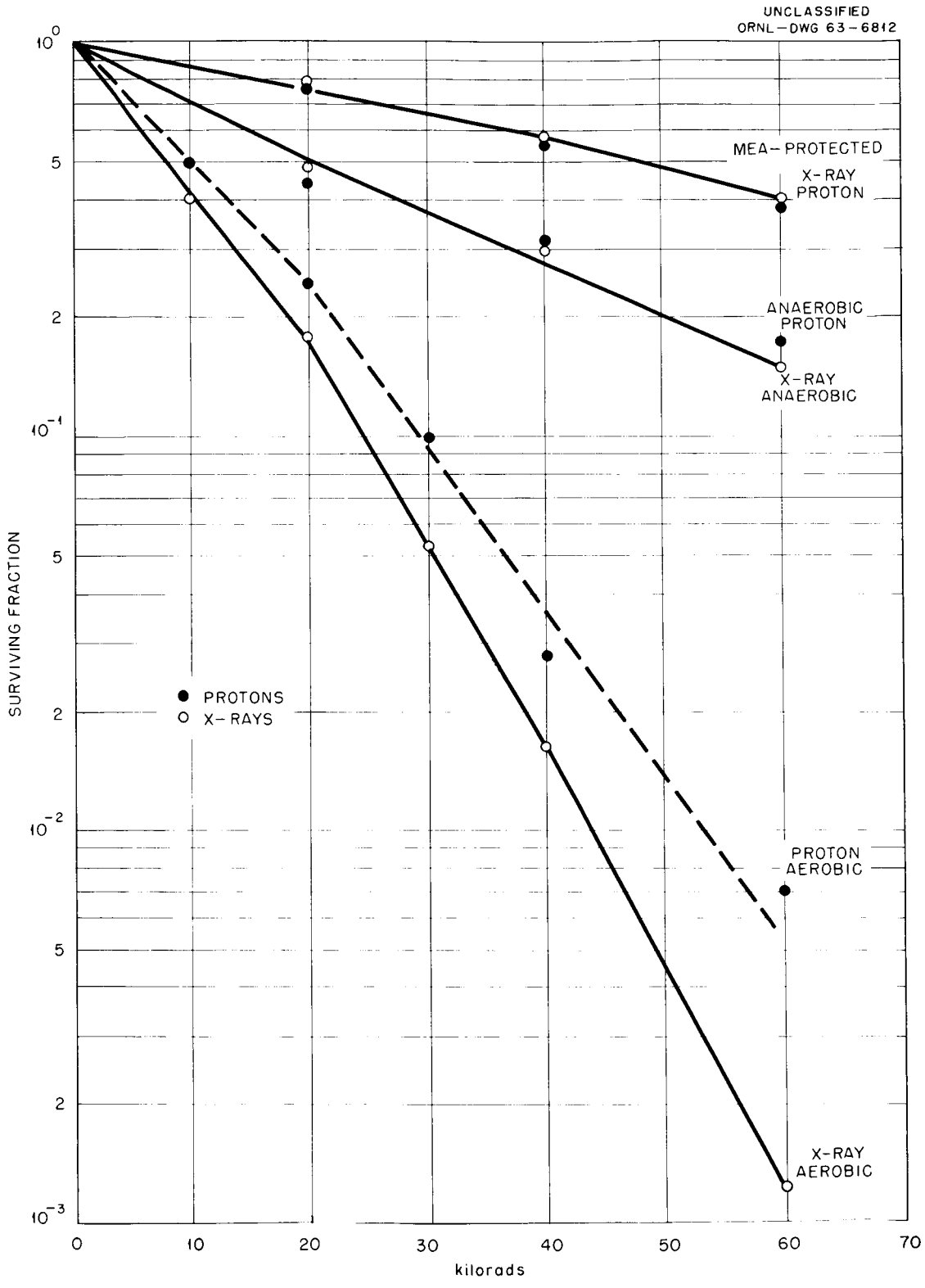

Fig. 7. Inactivation curves for E. coli B/r (ORNL) with 130 Mev protons and 250 kvp X rays.

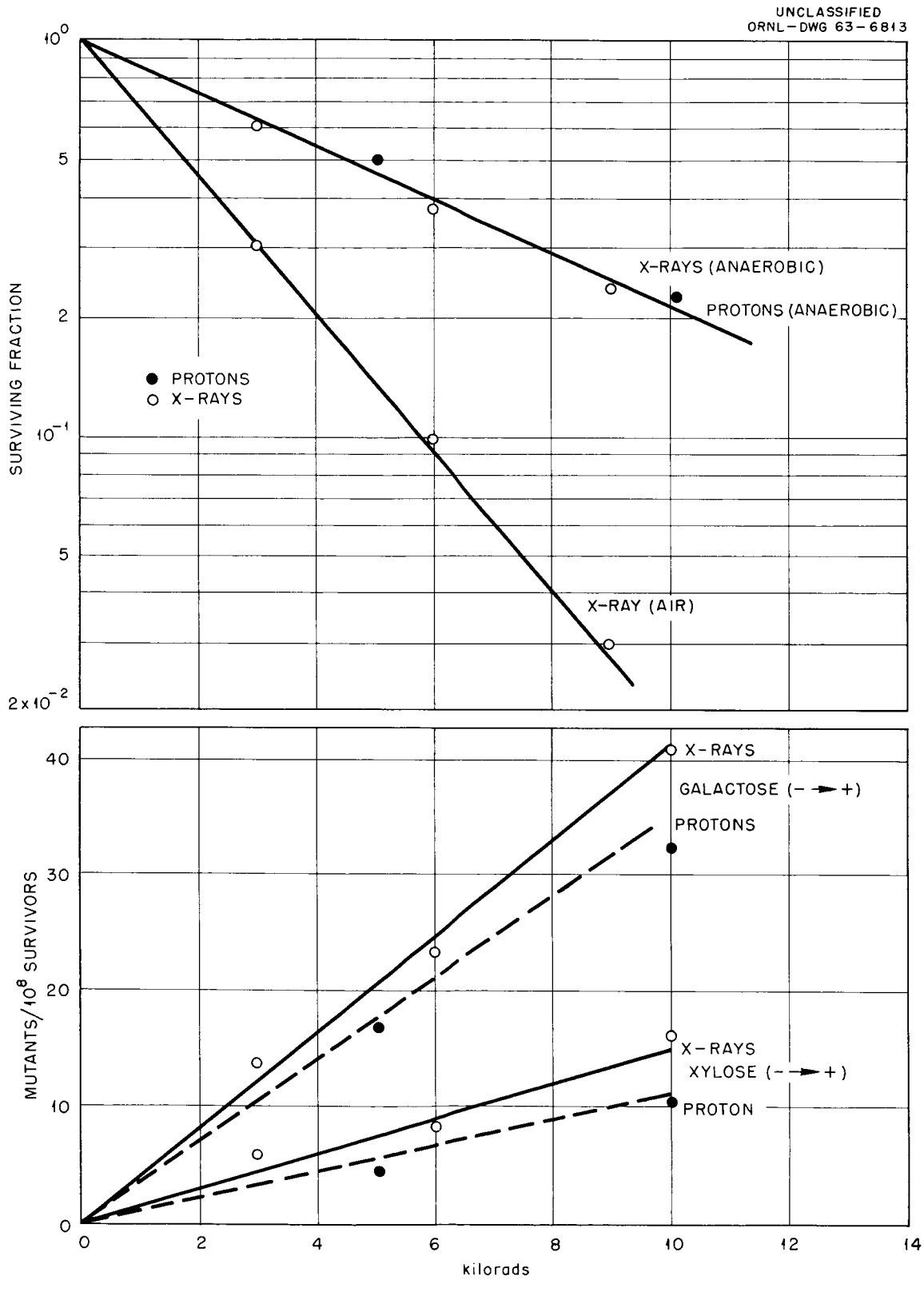

Fig. 8. Inactivation and mutation induction by 130 Mev protons and 250 kvp X rays. E. coli (AB-531).

Table 1. Experimental/Control Ratios of Normal Cells

Observed 72 Hours After Irradiation

Dose (rad)	Cell Type					
	Type A Spermatogonia[a]		Type B Spermatogonia[b]		Preleptotene Spermatocytes[c]	
	130-Mev Protons	X Rays	130-Mev Protons	X Rays	130-Mev Protons	X Rays
5	0.85	0.89	1.013	0.93	0.99	0.93
10	0.97	0.75	0.96	0.89	1.005	0.85
25	0.73	0.60	0.81	0.71	0.72	0.64
50	0.50	0.34	0.43	0.17	0.33	0.13
100	0.32	0.20	0.08	0.02	0.01	0.00
200	0.18	0.12	0.01	0.00	0.00	0.00

[a]Irradiated and scored as type A spermatogonia.

[b]Irradiated as type A, scored as type B spermatogonia.

[c]Irradiated as late type A and early intermediate spermatogonia, scored as preleptotene spermatocytes.

Table 2. Experimental/Control Ratios of Normal Cells Observed 72 Hours After Irradiation

Dose (rad)	Oocytes	
	130-Mev Protons	X Rays
5	1.042	0.717
10	0.809	0.608
15	0.447	0.143

c. Chromosome Aberrations in Human Blood Cells.--Blood samples from a normal female donor were irradiated with 130-Mev protons at doses of 50, 100, 150, and 200 rad. Leukocyte cultures were prepared from the irradiated blood and a control sample. A mitotic inhibitor was used to arrest the cells in metaphase and the cultures were fixed and mounted at 72 hours. The chromosome aberrations observed are given in Table 3.

A comparison of the coefficients of aberration production for 130-Mev protons and previously determined coefficients for 250-kvp X rays shows no significant difference between the values obtained for the two types of radiation. The proton data gives a coefficient of deletion production of 0.09×10^{-2} deletions/cell/rad, and a coefficient of ring and dicentric production of 0.60×10^{-5} rings and dicentrics/cell/rad^2. The coefficients of aberration production for 250-kvp X rays are 0.11×10^{-2} deletions/cell/rad and 0.52×10^{-5} rings and dicentrics/cell/rad^2. The RBE for 130-Mev protons in this system thus appears to be 1.

D. DISCUSSION

Several of the biological test objects have yielded an RBE of less than 1.0 for 130-Mev protons. Moreover, in the bacterial inactivation experiments an RBE of less than 1.0 was obtained for cells in equilibrium with air for 22-Mev protons as well. It is of interest that with the higher energy protons this value for RBE was obtained only for experiments done in air. Since the average LET of the protons of 130- and 22-Mev differ by a factor of 5 or less it is difficult to attribute the low RBE to linear energy transfer. It is also difficult to attribute the low RBE to systematic error in dosimetry for the various biological samples, since the same RBE for bacterial killing was obtained for protons of both energies and since the results for the human leukocytes did not show a low RBE for 130-Mev protons. It seems more likely that the reduced effectiveness may be attributable to a dose-rate effect. This would not involve the usual consideration of average dose rate (i.e., rads/min) but rather the high dose rate during the individual pulses, which are of microsecond duration. It is true that these pulses occur with greater

Table 3. Chromosome Aberrations Induced in Human Blood by 130-Mev Protons

Dose (rad)	Cells Scored	Chromatid Aberrations	Chromosome-Type Aberrations					
			Deletions	Rings	Dicentrics	Exchanges	Breaks[a] (%)	Rings and Dicentrics (%)
Control	100	1	0	0	0	0	0	0
50	100	0	4	0	3	2	10	3
100	100	1	12	0	3	2	18	3
150	100	0	18	0	6	2	30	6
200	100	0	21	2	23	5	71	25

[a]Deletions + twice rings and dicentrics; exchanges are excluded because of the subjective nature of their determination.

frequency than do those from a self-rectifying, alternating current X-ray tube, but this might not compensate for the high output during the pulse from a cyclotron.

It is interesting to consider why the lower RBE for bacteria (0.8 as compared with X rays) is observed only in aqueous systems and under those conditions in which oxygen is present. The results are highly suggestive that dose-rate phenomena (high dose rate) are restricted to oxygen-mediated indirect effects. It would be of interest to attempt irradiations with highly pulsed versus the usual X-ray irradiations to test this hypothesis.

The reduced biological response raises a question about the dosimeter responses at high dose rates. In general, however, one would believe that the dosimeter response would be reduced at very high dose rates, and this possible source of error is in the wrong direction to explain the results obtained.

The results to date are otherwise consistent with expectations, primarily based on data from microbiological systems, for which the major changes in RBE occur with LET 40-100 times higher than that for the 22-Mev protons.

III. IN-FLIGHT EXPERIMENTS

A. BIOLOGICAL MATERIALS AND METHODS

Most of the biological systems proposed for, or used for, actual in-flight experiments are the same as those already described in Section II. These include: bacterial inactivation and mutation, inactivation and mutation in the mold Neurospora, killing of spermatogonia and oocytes in the mouse, and chromosome aberration production in human leukocytes. Two additional experiments for in-flight use are (1) mutation in the wasp Habrobracon, and (2) chromosome aberrations in the Chinese hamster.

1. Mutation in Habrobracon

Males of the parasitic wasp Habrobracon normally develop from unfertilized eggs and are haploid. Females develop from fertilized eggs and are diploid. Thus in every second generation the entire chromosome complement of the organism can be examined genetically in the haploid state.

In the proposed experiments, Habrobracon males will be recovered after flight and mated to virgin females. Both egg hatchability and adult survival will be scored. Adult females will be bred unmated to determine the frequencies of recessive lethal mutations plus translocations by again scoring the frequency of females having a low hatchability and adult survival among their progeny. Several sons from each F_1 female exhibiting low hatchability will be outcrossed to females, and daughters from these crosses will be tested for low hatchability. The frequency of females showing low hatchability will be used to estimate the frequency of translocations. By this F_4 testing procedure almost the entire induced genetic damage to the genome can be evaluated; the rates of dominant and recessive lethal mutations, of visible mutations, and of translocations in the sperm of the treated Habrobracon males are thus determined.

2. Chromosome Aberrations in the Chinese hamster

The Chinese hamster, Cricetulus griseus, is a particularly valuable species for the investigation of radiation effects on mammalian chromosomes. It is available in inbred lines, which are maintained in the colony at the Biology Division of the Oak Ridge National Laboratory as well as elsewhere. The animals are small (25-30 g) and have about the same life-support requirements as mice. The chromosome number ($2n=22$) is very low, and the karyotype is well differentiated, offering a great advantage in speed and ease of scoring for chromosomal abnormalities. The only disadvantage of the Chinese hamster is the necessity for hand-breeding individual pairs; a great deal of effort is consequently required to maintain the inbred lines and to produce experimental stock.

A number of techniques will be used to determine the aberration frequencies in test animals. Both the bone marrow and the corneal epithelium will be used to prepare direct somatic mitoses suitable for scoring without any intervening tissue culture step. Techniques are also being developed to enable us to prepare mitoses from short-term cultures of peripheral leukocytes. Although this method does make use of a tissue culture step, it is believed that all of the leukocytes examined by this method will have been in their pre-DNA-synthesis phase during exposure, as they are in the human leukocyte system. Since this phase in the cell cycle is known to be one of very uniform sensitivity, it is anticipated that the peripheral leukocyte system will be of particular value. The examination of germ-cell meiotic figures is also being studied as a possibility for inclusion in the Chinese hamster measurements. Preliminary studies indicate that this technique may be feasible; if so, it will provide a direct measure of induced damage to cells in the germ line and thus of possibly inheritable genetic damage.

B. PHYSICAL METHODS

Three sets of experiments will be considered: (1) The series of two ballistic flights known just as "NERV II" and later as "BIOS I",

(2) the high-altitude balloon flights made from Goose Bay, Labrador, in 1962, and (3) the proposed "Radiation Biosatellite" flights.

1. BIOS I

The BIOS I flights were designed to carry certain physical and biological experiments into the inner Van Allen belt and to provide a reasonable period of weightlessness. A small recovery vehicle (RV) was launched from Point Arguello, California, on a ballistic trajectory calculated to reach a maximum altitude of approximately 1,200 miles, using an Argo D-8 four-stage, solid-fuel launch vehicle. The BIOS I RV was designed to provide about 28 minutes of weightlessness after "despinning" and before re-entry. The biological specimens, dosimeters, and a micrometeorite detection device were located within the recovery vehicle; also included were various tracking and recovery aids designed to facilitate its location and recovery from the sea.

The Biology Division samples included samples of bacteria, Neurospora, and human blood. Two members of the Biology Division staff were present at the launch site. They prepared the test material and loaded it into sterilized nylon and teflon sample holders designed and fabricated by the NASA Ames Research Center in preparation for the flight. Unfortunately, the design of the Recovery Vehicle was such that the biological experiments had to be turned over to the launch personnel approximately 12 hours prior to the scheduled launch time. Facilities for preparation of the biological samples were provided in a NASA van-type mobile laboratory, which proved to be a little cramped but adequate for the purpose. Communications, however, between the launch personnel and the biologists in the van (which was located some distance from the launch site) proved to be inadequate; this led to considerable confusion and wasted effort.

One Biology Division staff member was sent out with the Naval Recovery Force, which consisted of two land-based, radar tracking aircraft, a radar missile-tracking ship, two destroyers, and a seaplane tender carrying a helicopter. A U. S. Navy Pacific Missile Range van-type mobile laboratory was provided aboard the seaplane tender for the completion of experiments

with the biological test materials after the RV had been picked up. Although a number of technical difficulties developed in this mobile laboratory, it was generally adequate for the purpose; in fact, a number of control experiments were successfully completed while the recovery force was at sea.

The first of the BIOS I vehicles was launched on November 15, 1961. Malfunction of the tracking system and of the radar-computer data link prevented determination of the impact point for many hours after launch. The recovery force operated efficiently, but no recovery could be made in the absence of impact-point data. A second launch was made 3 days later. Unfortunately, malfunction of the launch vehicle resulted in the loss of this RV also; tracking data indicated that the trajectory caused the RV to burn up on re-entry into the atmosphere.

2. Goose Bay Balloon Flights

Biology Division experimental materials were flown on three of the five high-altitude balloon flights made from Goose Bay, Labrador, in July and August of 1962. The flights were designed to achieve an altitude of approximately 130,000 feet and to travel west at a latitude of 53°N for about 2 1/2 days. They were tracked by an aircraft equipped to cut the payload loose from the balloon by radio command and to track the payload as it returned to earth on a parachute. Recovery was possible by a light plane on floats, by helicopter, or by a party on foot, depending on the terrain in the landing area.

The Biology Division materials flown included individual Chinese hamsters and samples of bacteria and Neurospora. In addition, blood samples were to be obtained from the Rhesus monkeys flown. One Biology Division staff member was present at the launch site to prepare the animals and to help with the insertion of the bacteria and Neurospora samples. Another staff member was stationed at the NASA Ames Research Center at Moffett Field, California, to which the biological materials were to be flown immediately upon recovery.

No laboratory facilities were provided at the launch site for the preparation of fresh samples of bacteria and Neurospora. The specimens were therefore "prepackaged" in Oak Ridge and flown to Goose Bay a few days before the scheduled launch date. They were prepared in such a manner as to provide the maximum length of time before age made them unusable. The monkeys, which were provided by the Armed Forces Institute of Pathology, and the Chinese hamsters, which were flown to Goose Bay from the Oak Ridge National Laboratory Biology Division's colony, were provided with quarters on the U. S. Air Force Base at Goose Bay. Through the cooperation of the Base Medical Officer it was possible to obtain the use of the Base Clinical Laboratory facilities for the preparation of pre-flight leukocyte cultures from the monkeys.

After a number of technical difficulties and delays due to bad weather, the first balloon launching was quite successful. Because of weight considerations the part of the payload containing the bacteria and Neurospora was not included on the first flight. Some Chinese hamsters and two monkeys were flown, however. An apparently successful flight of about 2 1/2 days was made, but after recovery it was discovered that the animals had died early in the flight because of the failure of their life-support system.

The second flight was made several weeks later, the interval between flights being spent in correcting the life-support system difficulties.

On the second flight, as on the first, the only materials of interest to the Biology Division were the monkeys and the Chinese hamsters. After another successful flight the monkeys were recovered alive, but the hamsters died during the recovery operation. Since a change in plans had resulted in the use of monkeys from which no pre-flight cultures had been made, no post-flight cultures were attempted.

The cause of the hamsters' death has not been determined definitely, but it appears to have been due to the repeated innoculation of the animals with influenza virus prior to the flight. These innoculations had been made to permit a NASA staff investigator to carry out an experiment without having to fly additional animals; in retrospect this appears to have been a mistake.

One of the subsequent flights carried the bacteria and Neurospora. The samples were recovered and returned to Oak Ridge, but unfortunately so much time had elapsed since their preparation that the low survival levels precluded any useful measurements.

3. Proposed Biosatellite Flights

The ORNL Biology Division has participated in the early conceptual design work on the proposed NASA Radiation Biosatellite flights. These flights are designed to test the possibility of interactions between radiation and any of the other parameters of space flight, such as shock and vibrational loads and weightlessness. The satellites will carry their own radiation sources and as wide a variety of standard radiobiological test systems as possible, including at least five series of experiments proposed by the ORNL Biology Division. A small amount of design and feasibility work has been done to fit the proposed experiments to a preliminary satellite configuration and flight plan.

The experiments are designed for a roughly conical RV approximately 1 meter long and 1/2 meter across the mating face, which could weigh 100-150 kg. The satellite will travel in a peri-equatorial, circular, low earth orbit with a perigee of 100-200 km, for one day. The RV, with the experimental materials and environmental records would be recovered at sea and the biological materials placed in the experimenter's hands within a few hours after the recovery. The satellite would be "spin-stabilized" at launch and will thus have to be "de-spun" after injection into orbit and stabilized during flight so that the experiments will not be subjected to accelerations greater than 10^{-5} g.

The experiments will receive known doses of radiation of known quality during the "weightless" phase of the flight. The source of radiation will be a radioisotope which provides one gamma ray energy in excess of 0.5-Mev. The radioisotope source will be enclosed in a shield located at the base of the RV and provided with a shutter mechanism which when opened will permit irradiation of an axial, conical segment of the RV. A minimum of 2 hours of orbital flight will be provided both before

and after the irradiation takes place. The biological specimens will be located within the irradiated cone, located at various distances from the source so as to receive the required doses. The time of irradiation will be short enought to provide a minimum dose rate of 0.5 r/min. Fortunately, the samples requiring the highest doses are also the smallest physically, permitting an economical spatial array within the irradiated cone. The maximum to minimum dose ratio required by the experiments is compatable with a cone height of approximately 1 meter. The control specimens will be located in the shielded section around the source shield at the base of the satellite. The dose reduction factor (DRF) in the shielded section will be at least 10^{-5}. Preliminary calculation shows that it is impractical to provide controls shielded against both the source and the ambient radiation during the flight.

The satellite will carry a radiation spectrometer using a "Rossi chamber" as well as a nuclear track film pack, several tissue-equivalent total dose chambers, and several chemical dosimeters to characterize the ambient radiation received during the flight. Dosimetry for the irradiated specimens will be performed with films, silver metaphosphate glass rods, and possibly LiF thermoluminescent dosimeters. Prime dose determination will, however, depend on knowledge of the time sequence of operation of the source and on careful pre-flight measurements with the satellite in the laboratory.

Life support and environmental control will be provided for the specimens. The atmosphere will be 20% oxygen in nitrogen, at one atmosphere. The temperature will be maintained at 25°C and the relative humidity at between 40 and 70% during orbital flight.

Because of the nature of the experiments, little data need be obtained during the flight. Telemetry of data on spacecraft condition, life-support parameters and radiation source operation will be required, however, as well as periodic readout of the gamma ray spectrometer. Complete records of spacecraft condition, etc., can be recovered with the biological specimens. No experimental results will be obtained until after recovery of the specimens and subsequent work on them in the laboratory.

The biological specimens to be included in the Radiation Biosatellite from the Biology Division, and the measurements to be made on them are as follows:

1. Several containers of bacteria which will be exposed to doses of from 1000 to 10,000 rad; another container will be carried in the shielded part of the RV. After recovery of the containers the bacteria will be analyzed for both survival and mutation.

2. Four groups of "Millipore" filters with dried Neurospora conidia which will also be exposed to doses of from 1000 to 10,000 rad; a fifth group will be carried in the shielded section of the RV. After their recovery, the conidia will be assayed for survival and for ad-3 mutations, and a genetic analysis will be made of a representative sample of induced mutations from each exposure group.

3. Four groups of 20 Habrobracon males each which will be exposed to doses of from 500 to 4000 rad; a fifth group will be carried in the shielded section of the RV. After recovery the males will be tested for survival and for the induction of dominant and recessive lethal and visible mutations and of chromosomal translocations.

4. Four groups of five mice each, of which one group of males will be exposed to 50 rad and one group of females will be exposed to 15 rad. One group of males and one group of females will be carried in the shielded section of the RV. After the mice are recovered the testes and ovaries will be examined to determine the frequency of killed spermatogonia and oocytes.

5. Four groups of two Chinese hamsters each will be exposed to doses of 50, 100, 150 and 200 rads; a fifth group of two Chinese hamsters will be carried in the shielded section of the RV. When the animals have been recovered, the bone marrow and the corneal epithelium, and possibly also the peripheral leukocytes and germ cells will be examined to determine the rate of induced chromosomal aberrations.

6. Four groups of two 5 ml human blood samples will be exposed to doses of 50, 100, 150 and 200 rads. A fifth group of two blood samples will be carried in the shielded section of the RV. When the samples are recovered they will be analyzed for induced chromosome aberrations.

In all cases the results for the samples exposed in orbit will be compared with those for samples subjected to exactly the same treatment in a duplicate "satellite" in the laboratory.

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